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INTER-OFFICE CORRESPONDENCE

Richmond, Virginia CONFIDENTIAL

To: .Dr. C. K. Ellis Date: April 14, 1988

From: .R. D. Kinser

Subject: .TSNA Priority Program Operational Plans

OBJECTIVE: To design a reduced TSNA product by defining the mechanisms of TSNA pyrosynthesis and of TSNA transfer into mainstream smoke and examining various a priori methods of TSNA reduction. MS TSNA (TSNA/mg TPM) delivery of the desired product will be reduced 90% relative to the TPM corrected TSNA delivery of a 1987 full-flavored, blended cigarette. The target date for the laboratory model of a reduced TSNA product without flavor optimization is 1991. The desired product will be able to deliver a range of TPM consistent with current market needs.

STATUS AND BACKGROUND

Previous studies have indicated that mainstream (MS) TSNA arise from pyrosynthesis during smoking and transfer of filler (endogenous) TSNA into the smoke stream, and that the total delivery is also affected by some TSNA decomposition during the smoking process. Increased understanding of the formation of TSNA during curing has been obtained from two extensive curing studies, but current work aimed at reduction of TSNA transfer (distillation) is focused on selective removal of TSNA from cured filler. Of the solvents examined to date, the most efficient solvent for TSNA removal is ethanol, the most selective is hexane, and the best compromise for efficiency and selectivity is isopropyl alcohol. An optimized system which employs a mixture of ethanol and hexane has been developed. Also under investigation are ion exchange resins for removing TSNA from the extraction solvents. The possibility of reducing endogenous TSNA by biochemical alteration of tobacco, resulting in lowered biogenesis of alkaloids, is being examined.

Research on the inhibition of TSNA pyrosynthesis has indicated that the amine precursors of NNN and NAT are the secondary amines nornicotine and anatabine. The amine precursor of NNK has not been identified, but our research indicates that nicotine is not the primary amine precursor of MS NNK. Disproportionately high levels of MS NNK from base webs and base webs extracted with organic solvents suggest the possibility of a "bound" form of an amine, such as "unextracted nicotine", may be the NNK precursor. Experiments to investigate this hypothesis have been initiated. The addition of salts known to alter the NO content of MS smoke has been demonstrated to alter the MS TSNA delivery of both base web and burley, but in different ways. Preliminary results suggest that addition of nitrate to a filler may have a greater affect on pyrosynthetically generated TSNA (except NNK) than addition of alkaloid. Addition of cyanuric acid, a compound used to reduce the levels of nitrogen oxides in exhaust gases, did not lower MS NO or TSNA deliveries of a burley cigarette. Cyanuric acid also had no effect on the MS NO deliveries when incorporated into non-tobacco smoking materials at a

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variety of levels. Exposure of filler to NO did not result in increases in filler TSNA content. Model studies have indicated that antioxidants accelerate TSNA thermal decomposition; these studies are continuing.

STRATEGIES

These plans assume that Philip Morris chooses to not exert significant influence on tobacco cultivation, and therefore concentrate on tobacco treatment methods for decreasing TSNA delivery by distillation and methods which inhibit TSNA pyrosynthesis. Control of TSNA formation requires a greater understanding of those processes than currently possessed by us or described in the scientific literature, and the objective for the program incorporates the need for these fundamental studies. This basic research also includes a strategy designed to evaluate the possibility of TSNA reduction by biochemical alterations to the tobacco plant. The target date represents our best prediction for a development model meeting the 90% reduction goal using technologies and knowledge not available at this time. The priority assigned to each strategy, indicated by the number preceding the strategy, is based upon discussion with you and Dr. Sanders. With the exception of the strategy concerning oriental tobacco, this represents no significant deviation from the average of the priority assignments made by the scientists working in the program. Tactics will be designed for achievement of Strategy 9 as more information about various additives becomes available.

REDUCTION OF MS TSNA BY INHIBITING THE PYROSYNTHESIS OF TSNA

1. Reduce the levels of pyrosynthesized MS TSNA by removal of the amine precursor(s), or decreasing the reactivity to nitrosation of the amine precursor(s).

2. Reduce the levels of pyrosynthesized MS TSNA by incorporation into the cigarette design those aspects of oriental filler which result in an absence of significant TSNA pyrosynthesis from oriental tobacco.

3. Reduce the levels of pyrosynthesized MS TSNA by removing nitrosating agent(s) or precursor(s) of nitrosating agent(s), or blocking reaction pathways which form nitrosating agent(s) or which yield TSNA from the nitrosating agents.

REDUCTION OF MS TSNA BY REDUCING ENDOGENOUS TSNA IN FILLER

4. Reduce MS TSNA by selective removal of TSNA from filler.

5. Reduce MS TSNA by decreasing endogenous TSNA by biochemical alteration(s) to tobacco.

REDUCTION OF MS TSNA BY ENHANCING DECOMPOSITION OF TSNA

6. Evaluate the enhancement of TSNA decomposition during smoking as a method for reducing TSNA delivery.

REDUCTION OF MS TSNA BY ALTERING PHYSICAL/CHEMICAL PARAMETERS OF CIGARETTES

7. Reduce the levels of pyrosynthesized MS TSNA by alterations in cigarette construction parameters.

8. Reduce the levels of pyrosynthesized MS TSNA by manipulation of filler salt content.

9. Reduce the levels of pyrosynthesized MS TSNA by manipulation of additives typically used in cigarettes but missing from the reference cigarette.

TACTICS AND TIMETABLE

Outlined below are detailed plans for 1988 and an overview of work planned for 1989 and 1990. Timeframes given are best estimates possible at this time and represent updates based on review of this plan at the end of the First Quarter, 1988. No attempt has been made to allow time for possible analytical or instrumental problems; any schedule revisions needed due to these causes will be made on a quarterly basis. Serious problems were experienced with all of the gc/tea systems during the First Quarter, 1988, and plans have modified accordingly. These plans do not include investigations of methods modifications which have been assigned lower priority and which will be evaluated as time permits. Also, research areas which appear promising at this time may be found non-productive and therefore be eliminated. Similarly, results of the earlier studies will surely suggest tactics and possibly even strategies which have not yet been considered; these plans will be updated as ideas develop. In the original plan, possible requests from other priority programs were not listed below; completion dates for one study were adjusted so that one professional has been allocated time for support of these programs. In this revision some of the requests which have been received have been listed.

FIRST QUARTER 1988

Amine Precursor Strategy

Determine the effect of combining a competitive primary amine with a tobacco alkaloid and nitrosating agent in a model system and the effect on MS TSNA levels of adding a competitive primary amine or a non-alkaloid secondary amine	March 31
Evaluate the role of unextracted nicotine in TSNA pyrosynthesis by determining:	
SS TSNA from base web	Jan. 21
Base web alkaloids	Jan. 31
Effect on MS TSNA of addition of a combination of protein and nicotine to examine the hypothesis of physical entrapment as "structure" of unextracted nicotine	
Plans for experiments	March 15
Initiate work on confirmation of NNK in base web	Jan. 15

Oriental Inhibitor Strategy

Ascertain the existence of a chemical inhibitor in oriental tobacco to TSNA formation/pyrosynthesis by determining:	
Delivery of MS TSNA from Bu CEL and MT CEL on Bu base web vs. Bu CEL on Bu base web	March 15
MS TSNA from washed oriental and precursors	March 15
Minor alkaloids in oriental	Jan. 31
TSNA in MT SS	Jan. 21
The effect of "local" cultivation on chemistry of oriental tobacco:	
Determine appropriateness of abienol study samples for study of "local" cultivation of oriental	March 31

Nitrosating Agent Strategy

Initiate literature search relevant to nitrosation of secondary amines (conditions for reactions and structures of possible nitrosating agents)	Jan. 15
Complete study of nitrate on base web	Feb. 15
Continue development of model for nitrosation agent add-backs	on-going

Extraction of Endogenous TSNA Strategy

Establish optimum conditions for extraction of TSNA	on-going
Examination of ion-exchange resins for removal of TSNA from extraction solvents	on-going
Perform necessary studies in support of Sepracor program	on-going

TSNA Decomposition Strategy

Initiate literature search relevant to homolytic cleavage of N-N bonds	Feb. 15
Continue characterization of antioxidants as decomposition aids	on-going

Support to Other Priority Programs

Development of analytical techniques for VNA determination	March 1
Determine TSNA deliveries for 1R4F	March 1

SECOND QUARTER 1988

Amine Precursor Strategy

Evaluate the role of unextracted nicotine in TSNA pyrosynthesis by the following:	
Initiate study of unextracted nicotine and base web NNK	July 1
Select model(s) for physically entrapped nicotine studies	April 15
Initiate experiments using above models	May 17

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Confirmation of NNK in base web	on-going
Identify other alkaloids which may be precursors:	
Development of assay for PsON	July 1
Replicate Hoffmann experiments with ^{14}C-nicotine	July 1

Oriental Inhibitor Strategy

Determining the effect of "local" cultivation on chemistry-of oriental tobacco:	
Initiate efforts to cultivate oriental "locally" if needed	April 1
Determine levels of unextracted nicotine in oriental	June 30
Compare MS TSNA delivery from oriental + alkaloid + nitrate with those from similarly treated bright	June 15
Initiate Tactic #2 of Strategy 2 if warranted:	
Tactic #2: Determine the reasons oriental does not yield significant levels of pyrosynthesized TSNA by evaluation of the following:	
CEL fractions in model reactions	initiate May 1

Nitrosating Agent Strategy

Complete literature search; write detailed proposal on nitrosation studies	July 1
Continue development of model for nitrosating agent study	on-going
Test organic-nitrite complex as nitrosating agent	June 15
Complete development of TEA measurement of puffXpuff NO	July 1

Extraction of Endogenous TSNA Strategy

Establish optimum conditions for extraction of TSNA	April 30
Complete development of process for extraction of TSNA and removal of TSNA from solvent as a concerted process	May 15
Perform necessary studies in support of Sepracor program	on-going
Evaluate samples from water adsorber for SCFE	May 31
Evaluate effect of photodissociation of NO-containing molecules	June 30?

Biochemical Alterations to Tobacco Strategy

Harvest tobacco root material	on-going
Optimize classical chromatographic techniques	May 31
Obtain monoclonal antibody from Hazleton	May 31

TSNA Decomposition Strategy

Complete characterization of antioxidants as decomposition aids	May 15
Complete literature search on catalyst for homolytic cleavage; write proposal	July 1

Cigarette Construction Parameters Strategy

Initiate cigarette construction parameters study (Paper porosity, packing density, circumference)	April 30
Initiate preparation of other cigarettes for remainder of study	June 30

Support to Other Priority Programs

Support to FTR	April 12
TSNA content of waxes from Project ART	May 15
Samples from SCFE program	June 30

THIRD QUARTER 1988

Amine Precursor Strategy

Evaluate the role of unextracted nicotine in TSNA pyrosynthesis by the following:	
Extract green tobacco with SCF and cure	as SCF schedule permits
Complete study of base web and unextracted nicotine	Sept. 30
Continue study of "trapped nicotine"	on-going
Continue evaluation of other alkaloids and derivatives which may be precursors:	
Determine PsON levels in fillers	Aug. 15
Initiate study of polymeric secondary amine as precursor	July 1
Evaluation of MS TSNA from SCFE tobacco with depleted secondary amines (minor alkaloids)	Sept. 15

Oriental Inhibitor Strategy

Determining the effect of "local" cultivation on chemistry of oriental tobacco:	
Completion of "local" cultivation of oriental	Sept. 1
Evaluate MS TSNA delivery from an RL composed of oriental CEL and CEL from SCFE burley on Bu base web	Sept. 15
Continuation of Tactic 2 from strategy 2 (if warranted):	
Tactic #2: Determine the reasons oriental does not yield significant levels of pyrosynthesized TSNA by evaluation of the following:	
CEL fractions in smoking experiments	Aug. 1
Organic extract from oriental applied to filler	Sept. 1
Organic extract from MT CEL applied to filler	Oct. 1

Nitrosating Agent Strategy

Initiate implementation of nitrosation proposal	Aug. 22
Initiate study of scavengers for nitrosating agent	Aug. 15

Extraction of Endogenous TSNA Strategy

Testing extraction/TSNA removal/addition process	Aug. 31
Subjectives on cigarettes from extracted fillers	Sept. 30
Perform necessary studies in support of Sepracor program	on-going
Evaluate samples from water condensation from CO_2 from SCFE program	Sept. 30

Biochemical Alterations to Tobacco Strategy

Complete purification and screening procedures with the antibody and PMT assays	July 1
Obtain 60 μg of protein	August 1

TSNA Decomposition Strategy

Conduct smoking experiments with optimized antioxidant levels	Sept. 1
Initiate implementation of homolytic cleavage proposal	Sept. 15

Cigarette Construction Parameter Strategy

Continue evaluation of construction parameters	on-going

FOURTH QUARTER 1988

Amine Precursor Strategy

Evaluate the role of unextracted nicotine in TSNA pyrosynthesis by the following:	
Complete analyses of cured, extracted green tobacco	Dec. 31
Continue evaluation of other alkaloids and derivatives which may be precursors:	
Complete study of polymeric secondary amines	Dec. 31

Oriental Inhibitor Strategy

Determining the effect of "local" cultivation on chemistry of oriental tobacco:	
Analysis of samples from "locally" cultivated oriental	on-going

Nitrosating Agent Strategy

Initiate quantitative study of effect of nitrate	Oct. 1
Initiate protein study	Oct. 1
Continue study of scavengers for nitrosating agents	on-going
Continue studies outlined in nitrosation proposal	on-going

Extraction of Endogenous TSNA Strategy

Investigate photolysis of TSNA as means of removal from extracting solvents	
Using PM equipment	Nov. 15
Using commercially available thin-film reactors	Dec. 15
Perform necessary studies in support of Sepracor program	on-going

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TSNA Decomposition Strategy

Characterize decomposition pathway from kinetic/thermodynamic perspective	Initiate
Effect of altered burn temperature on decomposition	Dec. 31

Cigarette Construction Parameters Strategy

Continue construction parameters studies	Dec. 31

1989

Effect of removal of alkaloid/amines by various processes on MS TSNA
Effect of trace metal content on MS TSNA delivery
Effect of pH of oriental
Complete protein and nitrate study
Initiate studies of role of NO in nitrosation
Completion of nitrosating agent studies
Evaluation of salt effects
Complete construction parameters studies

1990

Construction and evaluation of models based upon studies to date

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RESOURCE ALLOCATIONS FOR 1988

How are the personnel assigned to this program allocated?

Amine Precursor Strategy:
Haut 65%
Warfield 15%
Kaiser 55%
Lambert 10%
Kinser 15%

Oriental Inhibitor Strategy:
Morgan 5%
Haut 15%
Warfield 20%
Kinser 10%

Nitrosating Agent Strategy:
Morgan 50%
Kaiser 15%
Haut 10%
Kinser 10%

Extraction of Endogenous TSNA Strategy:
Warfield 60%
Tickle 25%
Lambert 15%
Kinser 10%

Biochemical Alteration of Tobacco Strategy:
Nakatani 50%
Dunn 100%
Malik 100%
Mooz 70%
Sherwood 100%
Sykes 100%

Decomposition of TSNA Strategy:
Morgan 35%
Tickle 35%
Kinser 10%

Cigarette Construction Parameters Strategy:
Lambert 60%
Kaiser 15%
Kinser 5%
Morgan 10%

Adjustments to Filler Salt Content Strategy: None in 1988

Are there enough people allocated to this program?

Project 6908 Activities:

At the end of 1987, the number of people allocated to this program was decreased when Kathy Hansen was temporarily assigned to the Project Delta priority program. Since it was predicted that her involvement in Project Delta would continue for at least six months, the plans outlined in this document do not include any contributions from Ms. Hansen, and do not represent the rate of accomplishment that would be possible if Kathy were participating in this work. While an additional Associate Scientist did join the project in mid-October, this individual has little experience with

chemical analysis, will not be completely trained until the end of first quarter, 1988, and will probably require close supervision for at least one other quarter. Ms. Hansen's skills in performing accurate analyses, interpreting the data, and communicating the results enabled her to independently perform much of the TSNA support function. This in turn permitted us to provide a high level of support to other R&D programs while continuing the more fundamental research designed to achieve our primary goal of MS TSNA reduction. Obviously, even strict adherence to the plans prepared assuming Ms. Hansen's absence will result in a slower rate of progress than would be achieved if Ms. Hansen, who has two years of research experience in this area, were participating in nitrosamine research. A support request from a high priority special program has already been received which requires pushing forward estimated completion dates for some of the 1988 plans. The timetable prepared also eliminated any improvements to the analytical methods currently employed, since this work is typically assigned a lower priority and there were no professionals available to conduct such studies. Due to the uncertainty in length of Ms. Hansen's current assignment, allocation of another professional to this program for the time of her absence would ensure that nitrosamine support functions are performed in a timely manner and that the nitrosamine research program timetable outlined in this document is met. Therefore, I recommend assignment of either an Associate Scientist A or B to this program for the duration of Ms. Hansen's involvement with the Delta priority program.

Also, there is only one full-time technician currently supporting the work of five professionals; until third quarter, 1987, there were two technicians. A technician already in the group is being trained to assist in nitrosamine analyses, but this individual is allocated primarily to the Lowered Biological Activity program and could be utilized less than 20% of the time. An increase in technician support of this program by addition of one Research Lab Technician III is needed.

When these plans were prepared in January of this year, information as to the number of TSNA analyses needed by other priority programs was not readily available. As the year has progressed, several requests have been received from two other priority programs and our colleagues at FTR R&D. Research addressing one strategy as been postponed an entire quarter because there are not enough people currently assigned to the TSNA program to conduct the planned research and address the unplanned support requests.

Project 1904 Activities:

A biochemist with experience in immunology research is needed in addition to the personnel already assigned to this program.

Are the people allocated to this program the right people in terms of skills?

In terms of skills, the current staffing of this program appears adequate through 1989, with one exception. Four senior professionals with formal training and years of research experience in organic chemistry and analytical chemistry are assigned to this program in 6908. Three (including Ms. Hansen) junior professionals and one technician, all with significant on-the-job training in the appropriate analytical methods and all assigned to Project 6908, capably assist in the fundamental research programs and

perform analyses requested by colleagues in other parts of R&D. Four senior professionals and one junior professional with research experience in several biiological disciplines are involved in the Project 1904 studies of biochemical alterations of tobacco. Additional expertise in biochemistry/immunology is needed in this area.

Are there special equipment and facilities and/or outside expertise required?

Other than the specialized analytical equipment already in our laboratories, the only other special equipment we foresee needing is available in the supercritical fluid extraction facility in the Physical Research Division and the greenhouse facility in the Chemical Research Division. Outside consultants in the area of nitrosation chemistry and/or N-N bond cleavage may be needed; the decision is dependent upon the results of literature searches planned for 1988. A contract with Hazleton Biotechnologies Company has been obtained to facilitate the research on biochemical alterations to tobacco.

IMPACT ON OTHER AREAS BOTH WITHIN AND OUTSIDE R&D

Research described in this plan will require specific assistance from the Analytical Research Division, the Chemical Research Division, and the Physical Research Division. For 1988, requests to Analytical should require 0.25 to 0.5 man-months to complete. Extractions to be requested from the Physical Research Division's supercritical fluid facility during 1988 also should be completed within 0.5 man-months. Growth of tobacco plants needed for the biochemical alterations of tobacco research will require 0.6 man-year of support from the greenhouse staff. Also part of this study is a $32,000 contract of 6 - 9 months duration with Hazleton Biotechnologies Company. Replication of experiments describing the formation of NNK reported by scientists at the American Health Foundation will require an estimated six man-months of work by members of Chemical Research. Ultimately machine-made cigarettes will be required for evaluation, but that will probably not be until 1990. Assistance and training from individuals more skilled in cigarette design than anyone currently working in this area will be required when model construction and evaluation becomes the primary task of this group.

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